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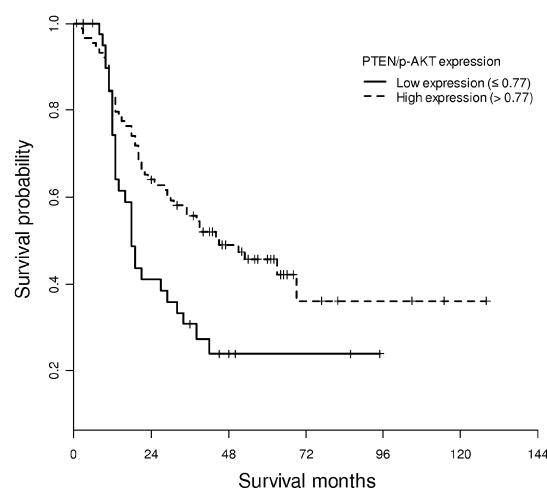
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(54) **Ratio based biomarkers and methods of use thereof**

(57) Compositions, methods and kits are described for identifying biomolecules (e.g., proteins and nucleic acids) expressed in a biological sample that are associated with the presence, development, or progression of a disease (such as cancer), or more generally determination of the etiology or risk factors associated with a disease. Sample types analyzed by the disclosed methods include but are not limited to archival tissue blocks that have been preserved in a fixative, tissue biopsy samples, tissue microarrays, and so forth. The methods disclosed herein correlate expression profiles of biomolecules with various disease types, and allow for the determination of relative survival rates; in some embodiments, the methods permit determination of survival rates for a subject with cancer.; In other embodiments, the disclosure relates to methods for evaluating therapeutic regimes for the treatment, such as treatment of cancer.

**FIG. 5A**





## EUROPEAN SEARCH REPORT

Application Number  
EP 13 18 0742

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	DOROTHEA BECKER ET AL: "Markers and Tissue Resources for Melanoma: Meeting Report", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US LNKD-DOI:10.1158/0008-5472.CAN-06-0921, vol. 66, no. 22, 15 November 2006 (2006-11-15), pages 10652-10657, XP007912895, ISSN: 0008-5472 * p.10655 col.1 last par. * -----	1-8, 11-13	INV. G01N33/574
A	CAMP R L ET AL: "Automated subcellular localization and quantification of protein expression in tissue microarrays", NATURE MEDICINE, NATURE PUBLISHING GROUP, NEW YORK, NY, US LNKD- DOI:10.1038/NM791, vol. 8, no. 11, 1 November 2002 (2002-11-01), pages 1323-1327, XP002406339, ISSN: 1078-8956 * p.1326 shaded box * -----	1-8, 11-13	TECHNICAL FIELDS SEARCHED (IPC)  G01N
X,P	JOON-YONG CHUNG ET AL: "The Expression of Phospho-AKT, Phospho-mTOR, and PTEN in Extrahepatic Cholangiocarcinoma", CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US LNKD-DOI:10.1158/1078-0432.CCR-08-1084, vol. 15, no. 2, 15 January 2009 (2009-01-15), pages 660-667, XP007912894, ISSN: 1078-0432 * the whole document * -----  -/--	1-8, 11-13	
<del>The present search report has been drawn up for all claims</del>			
Place of search <b>Munich</b>		Date of completion of the search <b>9 January 2014</b>	Examiner <b>Behrens, Ralf</b>
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... &amp; : member of the same patent family, corresponding document</p>			

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EPO FORM 1503 03/82 (P04C01)



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Application Number  
EP 13 18 0742

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	JOON-YONG CHUNG ET AL: "Tissue microarrays as a platform for proteomic investigation", JOURNAL OF MOLECULAR HISTOLOGY, KLUWER ACADEMIC PUBLISHERS, DO, vol. 38, no. 2, 5 September 2006 (2006-09-05), pages 123-128, XP019501281, ISSN: 1567-2387 * abstract; introduction; p.126 * -----	1-8, 11-13	TECHNICAL FIELDS SEARCHED (IPC)
A	BRAUNSCHWEIG TILL ET AL: "Tissue microarrays: bridging the gap between research and the clinic", EXPERT REVIEW OF PROTEOMICS, FUTURE DRUGS, LONDON, GB, vol. 2, no. 3, 1 June 2005 (2005-06-01), pages 325-336, XP009132948, ISSN: 1744-8387 * p.330 last par., p.331 col.2 * -----	1-8, 11-13	
A	JOON-YONG CHUNG ET AL: "Transfer and multiplex immunoblotting of a paraffin embedded tissue", PROTEOMICS, WILEY - VCH VERLAG, WEINHEIM, DE LNKD- DOI:10.1002/PMIC.200401343, vol. 6, 1 January 2006 (2006-01-01), pages 767-774, XP007912896, ISSN: 1615-9853 ----- -/--	1-8, 11-13	
<p>3 <del>The present search report has been drawn up for all claims</del></p>			
Place of search Munich		Date of completion of the search 9 January 2014	Examiner Behrens, Ralf
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... &amp; : member of the same patent family, corresponding document</p>			



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Application Number  
EP 13 18 0742

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	QIAO JINGBO ET AL: "Gastrin-releasing peptide-induced down-regulation of tumor suppressor protein PTEN (phosphatase and tensin homolog deleted on chromosome ten) in neuroblastomas", ANNALS OF SURGERY, J.B. LIPPINCOTT COMPANY, PHILADELPHIA, US LNKD-DOI:10.1097/01.SLA.0000161173.47717.71, vol. 241, no. 5, 1 January 2005 (2005-01-01), pages 684-692, XP009137772, ISSN: 0003-4932 * abstract; discussion p.689 col.1 *	1-8, 11-13	
X	TERAKAWA N ET AL: "Loss of PTEN expression followed by Akt phosphorylation is a poor prognostic factor for patients with endometrial cancer", ENDOCRINE-RELATED CANCER, JOURNAL OF ENDOCRINOLOGY LTD., BRISTOL, GB LNKD-DOI:10.1677/ERC.0.0100203, vol. 10, no. 2, 1 June 2003 (2003-06-01), pages 203-208, XP008116820, ISSN: 1351-0088 * abstract *	1-8, 11-13	
<p><del>The present search report has been drawn up for all claims</del></p>			TECHNICAL FIELDS SEARCHED (IPC)
Place of search		Date of completion of the search	Examiner
Munich		9 January 2014	Behrens, Ralf
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

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EPO FORM 1503 03.82 (P04C01)



Application Number

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**CLAIMS INCURRING FEES**

The present European patent application comprised at the time of filing claims for which payment was due.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due.

**LACK OF UNITY OF INVENTION**

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

2(completely); 1, 3-8, 11-13(partially)

☐ The present supplementary European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims (Rule 164 (1) EPC).



**LACK OF UNITY OF INVENTION  
SHEET B**

Application Number

EP 13 18 0742

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 2(completely); 1, 3-8, 11-13(partially)

A method of determining cancer prognosis according to claim 1 comprising PTEN and p-AKT as biomarker indicator.

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2-99. claims: 9, 10, 15(completely); 1, 3-8, 11-13(partially)

A method of determining cancer prognosis according to claim 1 comprising two biomarkers selected from those listed in claims 9-10, 15 (or those listed in the application on pp.24-38, in particular p.28 l.10 - p.31 l.20 and p.33 l.16 - p.37 l.20, or in the additional statements 1-42 on pp.89-95) as biomarker indicator.

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100. claim: 14

A method of determining relative cancer prognosis or survival rates for a subject with a solid tumor according to claim 14.

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